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Induction of suppressiveness to Fusarium wilt of chrysanthemum with composted sewage sludge

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ABSTRACT

The effectiveness of composted sewage sludge incorporated into *Pinus* bark-based substrate with or without biofertilizer, fish hydrolyzate, chitosan and *Trichoderma asperellum* was evaluated for the control of Fusarium wilt in chrysanthemum. The substrate was obtained from pots containing chrysanthemum plants killed by the pathogen. Half of the substrate was sterilized prior to the incorporation of sewage sludge (0, 10%, 20% and 30% v/v). These substrates were or were not supplemented with the following: biofertilizer, fish hydrolyzate and *Trichoderma*. The mixtures were transferred to pots, and the chrysanthemum was transplanted. For all treatments, half of the plants were sprayed weekly with chitosan. Assessment of severity was performed on the 8th, 12th, 15th and 20th week after transplanting. In the 12th week, microbiological and chemical analysis of the substrate was performed. The incorporation of composted sewage sludge into the *Pinus* bark-based substrate significantly reduced Fusarium wilt, which was progressively decreased as the concentration of sewage sludge increased. The addition of biofertilizer, fish hydrolyzate, chitosan and *Trichoderma* had no effect on the disease. The microbial community was greater in non-disinfested substrates. The results indicate that suppressiveness is related to the interaction of chemical and microbiological factors.

Key words: *Chrysanthemum morifolium*, biosolid, container media, organic matter, soil-borne pathogens

INTRODUCTION

The fungus *Fusarium oxysporum* f. sp. *crysanthemi* is the causal agent of wilt in chrysanthemum and, when present in the production system, may be responsible for losses of more than 50% (Pinto et al., 2010). This pathogen is difficult to control because there are no resistant varieties or registered fungicides. Furthermore, the pathogen produces chlamydospores that can survive for years in soil or substrate. *Fusarium* may also be present in irrigation water, in the pipe biofilms or in irrigation systems, further hindering its control. For chrysanthemums grown in pots containing substrates, the spread of the pathogen via water from the irrigation system is common. Therefore, to profitably produce plants, farmers must minimize the occurrence of the pathogen in the area by employing several management measures, such as the use of healthy seedlings, cleaning the irrigation systems, the use of a pathogen-free substrate and, if possible, the use of a suppressive substrate (Bettiol et al., 2009).

The substrates available on the market have general characteristics that make them conducive to *Fusarium*, such as acidic pH, low microbial activity and low electrical conductivity, among others (Santos & Bettiol, 2003; Amir & Alabouvette, 1993). Thus, even if the substrate is free of the pathogen, when infested by routes such as seedlings or irrigation water, the pathogen will encounter favorable conditions for its development. Therefore, suppressive substrates should be developed to maintain the disease at levels below the economic damage threshold (Baker & Cook, 1974; Bettiol et al., 2009).

An alternative for obtaining a suppressive substrate is the incorporation of organic materials in conducive commercial substrates, using the same principles described and studied for soil (Baker & Cook, 1974; Hoitink & Fahy, 1986; Bailey & Lazarovits, 2003; Termoshuizen et al., 2006; Zmora-Nahum et al., 2008). Several residues containing organic matter, when incorporated into soil or substrate, are able to support greater microbial activity, greater aeration and moisture retention, provide macro- and micronutrients, growth hormones and amino acids, induce host resistance, produce toxic compounds to the pathogen and make soil chemical characteristics unfavorable to the pathogen, thus inducing suppressiveness (Hoitink & Fahy, 1986; Hoper & Alabouvette, 1996; Alabouvette, 1999; Conn & Lazarovits, 1999; Tenuta et al., 2002; Bailey & Lazarovits, 2003; Abassi et al., 2004; Blum & Rodriguez-Kabana, 2004; Conn et al., 2005; Ureba et al., 2005; Termorshuizen et al., 2006; Yogev et al., 2006; Ghini et al., 2007; Zmora-Nahum et al., 2008; Lazarovits et al., 2009; Mendes et al., 2011). Specifically for the Fusarium-chrysanthemum pathosystem, a wood bark-based compost has been reported to induce suppressiveness (Chef et al. 1983). Pane et al. (2011) and Alfano et al. (2012) also provide evidence for induction of substrate suppressiveness in other pathosystems.

Organic materials have particular characteristics that may or may not make the substrate suppressive to a particular pathogen (Termorshuizen et al., 2006). The induction of suppressiveness to Fusarium wilt by means of incorporating organic matter in the soil or substrate was demonstrated by Alfano et al. (2012) working with olive cake compost; by Salem et al. (2012) evaluating the compost produced from tree leaves and wood; by Ghini et al. (2007) and Bettiol et al. (2009) with sewage sludge; Bettiol et al. (2009) with fish hydrolyzate; Ureba et al. (2005) with chiken manure; Kouki et al. (2012) with compost from plant residues and *Posidonia oceanica*; and Yogev et al. (2006) with a mixture of residues.

The aim of the present study was to evaluate the suppressiveness of *Pinus* bark-based substrates supplemented with organic residues (composted sewage sludge, biofertilizer and fish hydrolyzate) and *Trichoderma asperellum* to control wilt caused by *Fusarium oxysporum* f. sp. *crysanthemi* in chrysanthemum. In addition, the induction of resistance in plants treated with chitosan on the aerial portion was evaluated.

MATERIAL AND METHODS

The experiments were performed with the hardy garden mum (known in Brazil as "bola-belga") chrysanthemum (*Chrysanthemum morifolium*) cultivar Papyrus White on a property specialized in chrysanthemum production in Holambra/SP, Brazil. Crop management was performed according to the producer's standard practices, and the plants were monitored for 20 weeks, from the planting of plantlets produced by the rooting of cuttings to the final evaluation. The plants were grown in plastic pots containing 3.3 L of substrate, with one plant per pot. In the first week after transplanting, irrigation was performed with water only and, during the following 19 weeks, via fertigation (0.5 g L^{-1} calcium nitrate; 0.6 g L^{-1} NPK 5-30-15; 0.6 g L^{-1} potassium sulfate; 0.25 g L^{-1} magnesium sulfate and 0.25 g L^{-1} ammonium nitrate). During the first 10 weeks (vegetative stage), the plants were maintained in an environment with a light regime of over 15 hours/day and were then transferred to an environment with a photoperiod of less than 12 hours/day (generative phase). Fertigation was automated and localized in the pot, and was performed three to four times per day.

The substrate used in the tests was obtained from pots containing the substrate Multiplant® (pH 5.5; EC 0.6 µS cm^{-1}) with chrysanthemum plants that were diseased or killed by *Fusarium*. In the experiments, no artificial infestation was performed on the substrates with *Fusarium* because the pathogen was recovered from the pots with diseased plants and because both the water and irrigation systems were also naturally infested with the pathogen. This infestation was verified by plating samples of plants and irrigation water as well as parts of the vases and irrigation systems on Komada's medium, along with microscopic observation of pathogen structures. The pathogen population was estimated as 5 x 10^3 CFU/mL of the substrate by plating on Komada's medium.

Half the volume of the substrate was disinfested using steam treatment for approximately 12 h at 80°C, and the other half was not disinfested. Subsequently, in a factorial design, composted sewage sludge with and without heat treatment obtained from the sewage treatment plant in Jundiaí/SP, Brazil was incorporated into the substrate at concentrations of 0%, 10%, 20% and 30% (v/v). These mixtures were or were not treated with biofertilizer (Machado & Bettiol, 2010) at 14 mL L^{-1} (Table 1) and a *Trichoderma asperellum* [SF04 (URM-5911) – Quality WG - Laboratório de Biocontrole Farroupilha Ltda] suspension with 10^8 conidia

TABLE 1 - Chemical and physical attributes of sewage sludge, biofertilizer and fish hydrolyzate added to the pine bark-based substrate for inducing suppressiveness to *Fusarium* wilt in chrysanthemum

ATTRIBUTE		Sewage sludge	Biofertilizer	Fish hydrolyzate
pH		4.5	4.9	-
N		26.2	0.7	11.5
P		10.7	0.1	23.0
K		1.7	0.1	-
Ca	g kg^{-1} or g L^{-1}	2.3	0.2	11.5
Mg		0.9	0.1	-
S		6.4	0.1	-
C		264.6	41.1	207
Fe		45.4	402.5	2.88
B		58.0	0.01	-
Cu	mg kg^{-1}	1058.0	15.0	-
Mn		82.4	7.5	0.58
Zn		123.4	30.0	-
Moisture	(%)	14.6	99.6	-
C/N ratio		10.1	58.7	18.0

mL^{-1} applied at 200 mL per pot. The substrates obtained were placed in 3.3 L pots for the subsequent planting of chrysanthemum plantlets. After transplanting, half of the pots in each treatment had their aerial portions sprayed weekly with chitosan at a concentration of 200 mg L^{-1} for 10 weeks. A second similar experiment was conducted with the same treatments, replacing only the biofertilizer with fish hydrolyzate (Fishfertil® - Table 1) at 10 mL L^{-1}.

Assessment of Fusarium wilt severity was conducted using the disease scale proposed by Pinto et al. (2010), in which 0 = healthy plant, 1 = plant with slightly darkened vascular tissues in the central stem, 2 = plant with fully darkened vascular tissues in the central stem, 3 = plant with the vascular tissues in the central stem fully darkened and at least one darkened vascular tissue in the secondary stem, 4 = plant with wilt symptoms and with all vascular tissues darkened and 5 = dead plant. Severity assessments were destructive and performed at 8, 12, 15 and 20 weeks after planting; five plants were evaluated for each treatment at every one of these stages. Thus, at every stage, the experiments contained 32 treatments with 20 replicates each, totaling 640 pots per test at every stage. To confirm the presence of the pathogen in the plants, plating was performed using stem fragments on Komada's medium for further observation of the fungal structures under a light microscope, and a pathogenicity test was conducted thereafter.

At 12 weeks after planting, the community of fluorescent bacteria and fungi present in the rhizosphere of the chrysanthemum plants was assessed. For this purpose, one-gram aliquots of the substrate from the rhizosphere, representative of each treatment, were collected and added to 9 mL sterile distilled water for performing serial dilutions to a concentration of 10^{-5}. In Petri dishes containing culture medium divided into quadrants, four 10 µL aliquots were deposited into each quadrant, corresponding to one dilution. The plates were maintained at 24±3°C. The evaluation was performed by counting the number of colonies originating from each drop after 24 h for bacteria and after three days for fungi. The medium used for the bacteria and fungi counts was King B and Martin, respectively. In the same week, the total microbial activity of the substrate was determined by the hydrolysis of fluorescein diacetate (FDA) (Boehm and Hoitink, 1992) and the chemical attributes of the substrates (pH, electrical conductivity (EC), macro- and micronutrients) with extraction 1:1.5 (substrate:water) (Sonneveld et al., 1994) were evaluated.

The experiments a complete randomized 4-factorial design [4 (sludge doses) x 2 (with and without biofertilizer or fish hydrolyzate) x 2 (with and without *Trichoderma*) x 2 (with and without chitosan)] with 20 replicates. The results of severity obtained at the four assessment dates were used to calculate the area under the disease progress curve (AUDPC) using the Sigma Plot 10.0 software. Statistical analyses of the data were performed using SAS software (SAS Institute). Statistical analyses of the FDA, microbial community of fungi and bacteria, pH and EC (Table 2) and chemical characteristics (Table 3) data were performed using SISVAR software (Ferreira, 2000). We compared the effect of disinfestation, and the addition of sewage sludge, associated or not with biofertilizer (B) and fish hydrolyzate (H) on chemical and biological characteristics of the substrates, by Tukey test (P>0.05). Interaction of effect of each factor level in the presence/absence of the other factors was studied. For dose of sewage sludge we performed regression analysis.

RESULTS

The incorporation of composted sewage sludge to the pine bark-based substrate significantly reduced Fusarium wilt in chrysanthemum (Figure 1). The AUDPC generally decreased as the concentration of sewage sludge increased. When biofertilizer, fish hydrolyzate, and *T. asperellum* where incorporated into the substrate either alone or in combination, disease severity (AUDPC) was not reduced. In the presence of composted sewage sludge, regardless of the concentration, none of the supplements provided further disease reduction. The application of chitosan to the aerial part of the plant had no effect on the severity of Fusarium wilt in the different substrates (Figure 1).

Heat-treated substrates showed significantly lower microbial activity than the untreated, regardless of the sewage sludge concentration, as noticed by analyzing both FDA hydrolysis and the fungal community (Table 2). The initial substrate disinfestation negatively influenced the induction of suppressiveness because there was an increase in AUDPC among plants grown in disinfested substrates independent of the other supplements incorporated (Figure 1). Biofertilizer, independent of heat-treatment, increase FDA hydrolysis (Table 2).

In the substrate supplemented with sewage sludge, a positive correlation was found between doses of sewage sludge and concentrations of calcium ($yH=3.2925 + 2.57425x$; $r^2=90.95\%$, and $yB=-3.33+4.0395x$; $r^2=88.56\%$), magnesium ($yH=3.445 + 0.73825x$; $r^2=96.24\%$, and $yB=3.3625 + 0.9125x$; $r^2=95.62\%$), sulphur ($yH=11.865 + 3.0065x$; $r^2=94.03\%$, and $yB=5.3825+4.3395x$; $r^2=90.20\%$), zinc ($yH=0.030875 - 0.009787x + 0.000669x^2$; $r^2=96.76$, and $yB=-0.22325 + 0.0443x$; $r^2=73.80$), and manganese ($y=0.02925 - 0.01295x + 0.000825x^2$; $r^2=94.52\%$, and $yB=0.05025 - 0.044225x + 0.003262x^2$; $r^2=98.29\%$) (Table 3). In the treatments with sewage sludge, nitrate-N generally prevailed. There were no consistent alterations in relation to the chemical characteristics of the substrates, sterilized or non-sterilized, treated with biofertilizer and fish hydrolyzate (Table 3).

DISCUSSION

The composted sewage sludge incorporated into the substrate was the main cause for the induction of

TABLE 2 - Electrical conductivity (EC), pH, microbial activity (fluorescein diacetate hydrolysis - FDA) and microbial community of the fungi and bacteria of the pine bark-based substrate sterilized (S) and non-sterilized (NS) supplemented with sewage sludge (SS), biofertilizer (B) and fish hydrolyzate (H) in the first and second experiments, respectively

Treatment	FDA (μg g^{-1} of dry substrate)		Fungi CFU (10^4 g^{-1})		Bacteria CFU (10^6 g^{-1})		pH		EC (dS m^{-1})	
	S	NS	S	NS	S	NS	S	NS	S	NS
Biofertilizer										
B	6.52	7.37	1.0	6.2	32.0	15.6	6.2	6.4	0.3	0.3
Control	3.16	7.73	0.2	5.9	6.9	7.0	5.9	5.8	0.3	0.3
SS 10% + B	5.98	6.74	1.2	6.0	14.0	21.0	6.0	5.9	0.4	0.4
SS 10%	4.47	6.33	1.2	5.9	5.2	23.7	5.4	5.0	0.6	0.8
SS 20% +B	4.98	5.87	1.3	6.0	8.0	25.1	5.3	5.3	0.6	0.6
SS 20%	4.61	6.78	1.2	6.7	6.2	25.5	5.2	4.6	0.9	1.8
SS 30% +B	6.34	5.60	2.1	6.7	12.0	24.9	5.0	4.6	0.8	1.1
SS 30%	4.89	5.44	1.8	6.6	5.2	24.2	6.2	6.2	0.3	0.3
Mean	5.12b	6.48a	1.25b	6.25a	11.19a	20.87a	5.65a	5.47a	0.52a	0.7a
CV	19.06		7.81		55.34		3.38		36.38	
Fish hydrolyzate										
H	1.31	3.51	1.2	5.92	3.2	6.1	6.4	6.6	0.2	0.2
Control	1.57	3.27	1.5	5.36	2.7	3.9	6.2	6.4	0.2	0.3
SS 10% + H	2.25	2.84	3.1	3.1	1.8	6.2	6.2	5.9	0.2	0.5
SS 10%	1.77	3.59	1.9	2.8	0.6	3.8	5.9	6.2	0.5	0.3
SS 20% +H	1.93	3.18	0.1	7.1	2.7	3.5	5.7	5.4	0.4	0.6
SS 20%	1.97	2.95	1.9	6.10	1.6	2.9	5.7	5.8	0.4	0.7
SS 30% +H	2.06	3.38	2.2	13.2	2.3	5.1	5.0	5.0	1.0	0.8
SS 30%	2.44	2.84	1.9	12.0	1.2	5.3	5.2	5.1	0.7	1.0
Mean	1.91b	3.20a	1.72b	6.95a	2.01b	4.60a	5.79a	5.80a	0.45a	0.55a
CV	17.06		64.48		29.00		2.80		30.24	

Within each characteristics of substrates S and NS, means followed the same letter are not significantly different (Tukey alfa at P=0.05).

TABLE 3 - Chemical characteristics of the pine bark-based substrate (Control-C), sterilized or non-sterilized by steam and supplemented with sewage sludge (SS) plus biofertilizer (B) or fish hydrolyzate (H) in the first and second experiments, respectively

Nutrient	B	C	10%SS +B	10% SS	20%SS +B	20% SS	30%SS +B	30% SS	H	C	10%SS +H	10% SS	20%SS +H	20% SS	30%SS +H	30% SS
								Non-sterilized (mg L^{-1})								
NO_3-N	0.8	0.3	4.9	1	7.5	2.8	4.5	9.5	0.3	0.6	0.1	9.2	2.6	1.7	1.8	11.3
P	8.4	5.6	7.3	3.2	5	1.2	3.9	0.8	4.6	5.9	2.6	2.5	1.7	1.5	0.7	1.2
Cl	1.1	2.1	3.6	1.1	3.6	1.4	2.8	1.4	0.7	2.1	2.5	1.1	0.7	<0.01	< 0.01	2.1
S	16.2	29.3	26.2	40	73.9	76.2	116.7	101.5	18.2	16.7	19.7	52.8	41.9	45.6	141.2	82.6
NH_3-N	1.5	0.8	0.6	0.6	1	0.5	1	0.7	0.9	1	0.6	0.3	0.9	0.1	0.1	0.6
K	12.8	12.3	12.3	12.3	19.3	8.9	19.3	17.6	12.1	13	10.1	19.1	13.5	14.7	18.8	17.9
Na	15.1	18.3	17.2	17.4	15.6	17.7	17.7	16.7	14.7	13.1	13.8	23.4	14	14.9	17.9	18.3
Ca	7.9	15.3	20	23.8	52.9	53	87.9	83.8	9.2	9.3	10.3	36.6	28.1	30.3	116.2	61.9
Mg	3.4	8.3	7.8	11	22.8	18.2	30.8	21.6	3.9	4.4	3.9	16.1	10.4	11.1	31.9	20.3
Mn	0.01	0.02	0.1	0.1	0.4	0.3	1.4	0.7	<0.01	0.02	< 0.01	0.1	0.04	0.05	0.7	0.5
Zn	0.03	0.02	0.02	0.04	0.2	0.3	0.6	0.8	0.01	0.02	0.02	0.05	0.05	0.03	0.4	0.3
								Sterilized (mg L^{-1})								
NO_3-N	1.8	1.2	6.5	4.7	14.6	6.9	13.1	10.4	1.5	0.7	2.2	3.9	3.7	9	14	8.6
P	13.4	3.8	6.5	2.5	4	1	1.9	0.6	5.1	4.8	2	2	1.3	2.1	0.9	1
Cl	4.6	3.2	2.1	5.7	9.2	1.4	2.1	3.2	2.8	1.8	0.4	2.5	3.6	1.4	1.1	1.4
S	18.9	21.8	18.8	44	85.7	71.1	244	143.3	16.7	22	57.6	22.4	77.6	47.9	95.8	125.7
NH_3-N	0.7	0.5	0.2	0.9	0.5	0.6	2.1	2.8	0.8	0.8	0.7	0.5	1.3	1.1	1	4.8
K	17.4	7.2	15.9	19.3	19.3	8	16.2	16.2	15.7	11.1	12.5	12.3	26.8	21	18.6	19.3
Na	16.3	17.9	11	21.3	14.9	17.2	27.5	20.6	14.4	15.6	18.1	12.1	15.4	22.7	14.9	21.3
Ca	14	12.1	718	28.3	76.9	55.9	232	134.6	9.7	13.2	36	16.5	53.2	56.3	81.2	102.5
Mg	5.9	5.4	5.8	12.2	23.1	20	50.7	25.8	3.8	5.4	17.1	5.8	19.3	21.7	25.7	31.5
Mn	<0.01	<0.01	<0.01	0.01	0.3	0.1	2	0.8	<0.01	<0.01	<0.01	<0.01	0.1	0.01	0.2	0.2
Zn	0.04	0.02	0.05	0.04	0.4	0.2	2.3	2	0.03	0.02	0.03	0.03	0.1	0.1	0.3	0.4

*B and Cu>0.01, and Fe 0.1 to 0.5 for all mixtures.

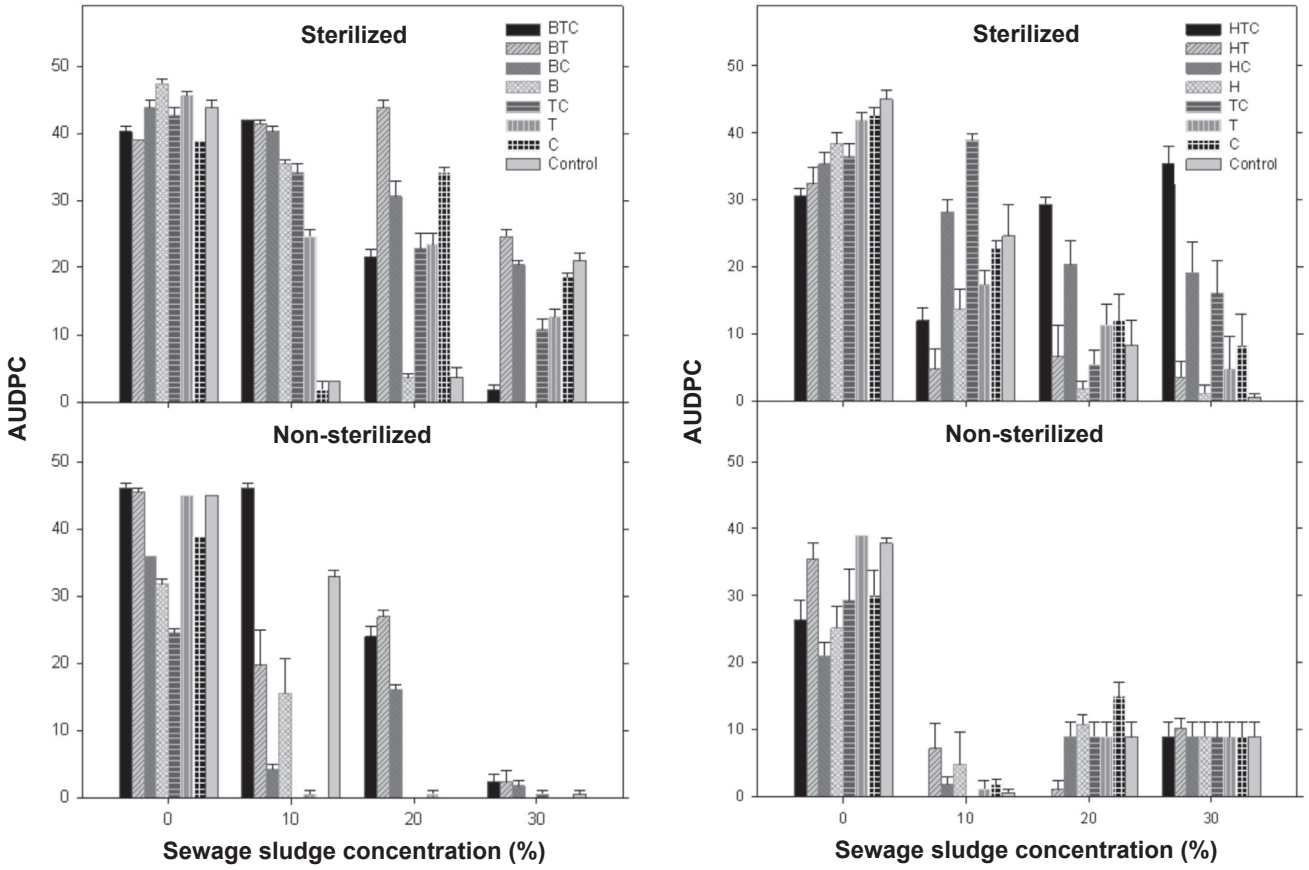

FIGURE 1 - Effect of the disinfestation of pine bark-based substrate (PB), which was naturally infested with *Fusarium*, on the induction of suppressiveness by composted sewage sludge in combination with biofertilizer (B), fish hydrolyzate (H), *Trichoderma asperellum* (T) and chitosan (C) on the area under the disease progress curve (AUDPC) of *Fusarium* wilt in chrysanthemum. The standard errors from each treatment of experiments 1 (B) and 2 (H) are presented.

suppressiveness against *Fusarium*, independent of the substrate disinfestation or supplementation with fish hydrolyzate, biofertilizer, *Trichoderma* or chitosan. These observations highlight the ability of composted sewage sludge to alter the biological, physical and chemical characteristics involved in the suppressiveness to *Fusarium*. Ghini et al. (2007) obtained similar results when studying the effect of raw (not composted) sewage sludge for the control of *Rhizoctonia solani* and *Ralstonia solanacearum*, whereas Santos & Bettiol (2003) and Cotxarrera et al. (2002) observed suppression of *Sclerotium rolfsii* and *Fusarium* wilt, respectively with composted sewage sludge.

The induction of suppressiveness with the incorporation of sewage sludge should be mainly attributed to biological factors because the microbial activity and fungal and bacterial communities were greater in the substrates not thermally disinfested, which also correlated with greater suppressiveness. The increased microbial activity induced by the incorporation of sewage sludge and other organic residues was also observed by Chen et al. (1988), Dissanayake & Hoy (1999), Cotxarrera et al. (2002), Santos & Bettiol (2003), Abbasi et al. (2004), Ghini et al. (2007) and Lazarovits et al. (2009).

Several authors have discussed the need to incorporate organic matter that stimulates the maintenance of microbial activity to obtain a substrate suppressive to soil-borne pathogens (Hoitink & Fahy, 1986; Chen et al., 1988; and Hoitink & Boehm, 1999). Visconti et al. (2010) found that the increased microbial activity in substrates treated with 20% fish hydrolyzate was responsible for the induction of suppressiveness to *Cylindrocladium spathiphylli* in *Spathiphyllum*. In addition to increased soil microbial activity, as determined by analyses of specific enzymes and soil respiration, the changes in the overall microbial community of the soil are also important. Postma et al. (2008) observed that some species of the soil microbial community are associated with suppressiveness, and this observation was verified by Lazarovits et al. (2009), who also found that the species composition changed with the incorporation of organic matter.

Associated with the soil microbial activity, the release of volatile fatty acids, both those present in organic residues and those released by the degradation of organic matter, is

also responsible for the induction of suppressiveness. This mechanism is amply discussed by Abbasi et al. (2004), Conn et al. (2007) and Lazarovits et al. (2009) and may also be associated with the incorporation of the sewage sludge compost, as there is a release of volatile fatty acids during sewage and sludge decomposition (Yang et al., 2012). In the substrate used, there is a possibility that this production of volatile fatty acids occurs throughout the crop cycle, as the sludge has a low carbon:nitrogen (C:N) ratio, while pine bark has a high ratio (Table 1).

Thermorshuizen et al. (2006) studied the suppressiveness of 18 substrate components against seven pathosystems and indicated that higher suppressiveness occurs when using a mixture rather than one component alone. The diverse composition of sewage sludge may have contributed to the observed results.

The heat treatment most likely eliminated the entire microbial community from the substrate, creating a biological vacuum, which did not occur in the non-disinfested substrate. Similar results, although in a study using fish emulsion, were obtained by Abbasi et al. (2004), who observed that pasteurizing the substrate resulted in a greater occurrence of *Rhizoctonia* in radish than in the unpasteurized substrate. These authors also found a reduction in the microbial community with the pasteurization of the substrate. These data demonstrate the biological nature of the suppressiveness induced by sewage sludge compost.

The aspects related to nutrition are of great importance regarding disease severity in plants (Engelhard, 1990). The substrate to which the sewage sludge was incorporated showed an overall increase in concentrations of nitrate-N, potassium, calcium, magnesium, sulphur and manganese, nutrients related to the occurrence of *Fusarium* in soils (Engelhard, 1990; Huber, 1994). In addition to the concentration, the form in which these nutrients are available is also important. Generally, the occurrence of diseases caused by *Fusarium* is reduced when nitrogen, which predominates in the soil, is in the form of nitrate-N and increased when present as ammonia-N. The form of nitrogen is related to pH, i.e., the disease is most severe when the pH is acidic and when ammonia is present (Huber, 1994). In treatments with sewage sludge, nitrate-N generally predominated. In the treatments with sewage sludge in which the pathogen was controlled, greater concentrations of sulfur and calcium were generally observed. Calcium is related to the integrity of the cell wall and membrane, which are physical barriers that the pathogen must face to penetrate and establish itself in the plant.

The electric conductivity (EC) values obtained did not explain the induction of suppressiveness by the sewage sludge, as there was no significant difference among the treatments. However, several authors found a correlation between the electrical conductivity of soil and suppressiveness to diseases (Amir & Alabouvette, 1993; Santos & Bettiol, 2003). Nevertheless, the importance of its variation along the cycle cannot be dismissed because the analysis of the EC was performed only halfway through growth.

In the present study, no reduction in the severity of *Fusarium* wilt was observed with the application of chitosan, a substance known to induce plant resistance to different pathogens in several pathosystems (Coqueiro et al., 2011).

As discussed and shown in the analysis, the induction of suppressiveness observed with the addition of sewage sludge to the pine bark-based substrate must not only be related to a single characteristic altered in the substrate, as there were significant changes in the microbial activity, nutrient concentration and pH of the substrate. Although not evaluated in the present study, evidences that changes in microbial composition and production of volatile compounds in the substrates probably contributed to the observed suppressiveness. These hypotheses are been investigated. The contribution of each factor alone in the induction of suppressiveness cannot be quantified using the findings of the present study. However, they constitute additional evidence of the efficiency of sewage sludge compost in controlling Fusarium wilt on chrysanthemum. Moreover, because the sewage sludge is rich in many macro- and micronutrients, it allows for the reduction in the amount of nutrients applied via fertigation.

Suppressiveness induced by composted sewage sludge was maintained through the last evaluation, i.e., up to the 20th week of plant development. This prolonged effect is an important practical aspect because plants are commercialized at 16 weeks, and at the end of the cycle due to intense flowering they become more susceptible to the pathogen. Thus, plants grown on substrates supplemented with sewage sludge were within the standard for commercialization until the end of their cycle (data not shown).

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